# Fabrication of cationized gelatin nanofibers by electrospinning for tissue regeneration

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#### 1. Cross-linking of Cationized gelatin nanofibers using glutaraldehyde

For comparison of the results, the CG nanofibrous mats were cross-linked using glutaraldehyde vapour. Cross-linking was carried out by placing 0.5 g of nanofibrous mats over a petridish containing 10 ml of 25 % glutaraldehyde solution (aqueous). The mats along with petridish was kept inside a sealed desiccator and allowed to cross-link with glutaraldehyde vapour for 72 h at room temperature. The glutaraldehyde cross-linked cationized gelatin (GT-CG) mats were then washed in double distilled water and dried under reduced pressure.

### 2. Morphology of GT-CG nanofiber mats

Figure S1 shows the SEM images of glutaraldehdye cross-linked CG nanofibers before and after swelling in water. SEM images reveal that, cross-linking and subsequent swelling process cause the fibers to lose its discreteness with increase in fiber diameter compared to as spun CG nanofibers. This may be due to the moisture present in aqueous glutaraldehyde. However, compared to as spun CG mats, GT-CG mats maintain the fibrous morphology even after dipped in water.



Figure S1: GT-CG mats (a) Before swelling and (b) after swelling in water

#### 3. MTT assay using L-929 fibroblast cells

L-929 cells are allowed to grow in the medium of the extract of the test materials, namely DA-CG and SA-CG. For comparison of the results, a control material, namely, glutaraldehyde cross-linked CG mats (GT-CG) is also used. GT-CG is prepared by exposing CG mats to glutaraldehyde vapours for 72 h. On comparing the results, cells in DA-CG and SA-CG extracts show increase in the percentage cellular activities during 1 to 3 days which in turn relates to the cellular growth and proliferation. On the other hand, cells in contact with the extract of GT-CG show retarded growth of L-929 cells, which can be due to the internal toxicity effects of the residual glutaraldehyde (Figure S 2)



Figure S 2: Percentage metabolic activities of L-929 cells in the extract of DA-CG, SA-CG and GT-CG nanofibers for 1 and 3

days culture